DNA methylation: **A molecular lock**

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In mammals, the promoters of expressed genes are generally unmethylated, whereas those of genes that are not expressed are methylated. Two recent papers help to explain the mechanism by which methylation modulates gene expression.

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Mammalian DNA shows a striking epigenetic modification — the methylation of cytosines, forming 5-methylcytosine, which occurs exclusively where a cytosine occurs immediately 5′ to a guanosine, forming what is commonly referred to as a CpG dinucleotide. Approximately 60–90% of the CpGs in the genome of an adult mammal are methylated in this way, a pattern which is set up during embryogenesis. In the blastula, most of the DNA is unmethylated; after implantation, a wave of *de novo* methylation modifies most of the genome except for the so-called 'CpG islands' — regions with very high densities of CpGs — which are associated with the essential, 'housekeeping' genes and which remain unmodified. Tissue-specific genes undergo demethylation only in the tissues in which they are expressed, creating a bimodal pattern of methylation which is maintained in the adult. Thus, a correlation is observed between hypomethylation of the promoter region and transcriptional activity [1].

DNA methylation is necessary for proper embryonic development, as mice lacking any functional DNA methyltransferase gene fail to develop properly and die in midgestation [2]. It has been suggested that methylation is a novel repression mechanism developed in more complex organisms to reduce 'transcriptional noise' [3] — the inappropriate transcription of a gene in cells where it is meant to be silent. One would expect such stochastic misfiring of a gene to be more of a problem the greater the number of genes, and this might be particularly important if the misexpression of a gene during crucial points of development is lethal. One can imagine that disrupting a global repression mechanism would have deleterious effects on the developing embryo.

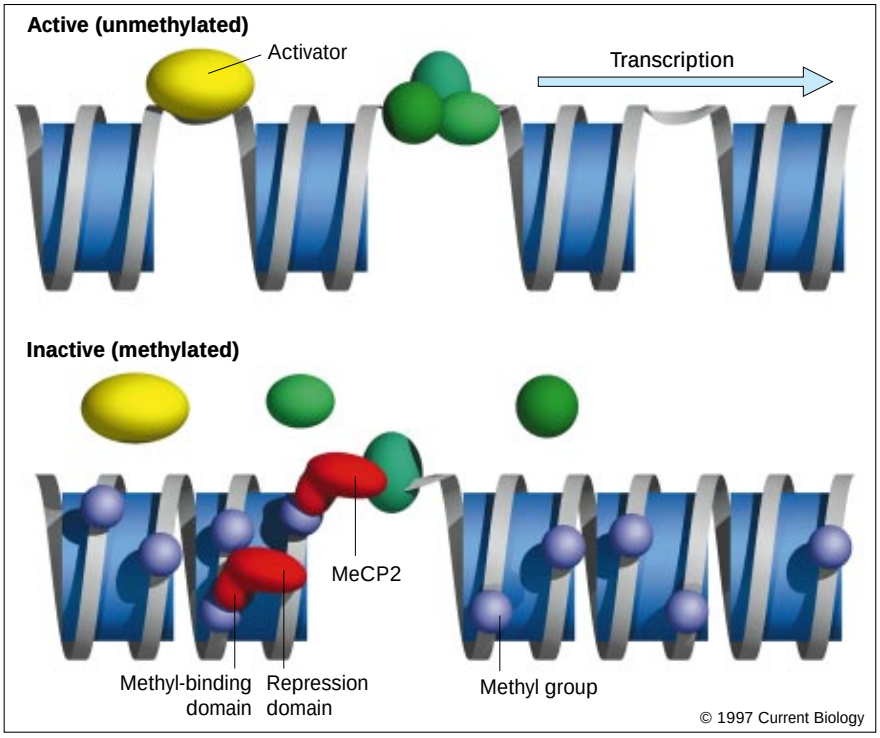
Several lines of evidence indicate that the relationship between methylation and gene expression is not just correlative. The methylation of genes *in vitro* prevents their subsequent expression in transfected fibroblasts [4]. In addition, the treatment of cells with 5-azacytidine, an inducer of demethylation, leads to the activation of several repressed endogenous genes [5]. These experiments and others have shown that methylation actually causes the repression of gene expression.

There appear to be three possible ways by which methylation can affect gene expression. One involves the methylated CpG residues interfering directly with the binding of specific transcription factors to DNA. Several transcription factors — AP-2, c-Myc/Myn, E2F and NFκB — bind to DNA sequences that include CpGs and have been shown to be sensitive to methylation at these sites. Some transcription factors, however, are not sensitive to methylation — examples are Sp1, CTF and YY1 [6]. Most of the factors that have been shown to be methylation-sensitive are ubiquitous. Methylation does not appear to interfere with the binding of gene-specific transcription factors, but rather to interfere with the binding of ubiquitous factors in cells where the gene is not expressed [7]. Methylation therefore works as a global mechanism of repression.

A second possibility is that the direct binding of specific factors to methylated DNA mediates repression. Two such factors, MeCP1 and MeCP2, have been identified and shown to bind to methylated CpG in any sequence context. MeCP1 binds to DNA containing multiple symmetrically methylated CpGs [8]. MeCP2 is more abundant than MeCP1 in the cell and is able to bind to DNA that is asymmetrically methylated, with just a single methyl-CpG. In addition, the distribution of MeCP2 on the chromosome parallels that of methyl-CpG [9]. Experiments on mice with a disrupted *MeCP2* gene have shown that MeCP2, like DNA methyltransferase, is dispensable in stem cells, but essential for embryonic development [10].

A new study by Nan *et al.* [11] has identified a repressor domain in the MeCP2 protein, which may explain its mode of action. It has previously been shown that the protein contains a methyl-CpG-binding domain of 80 amino acids which is essential for chromosomal localization [12,13]. Now, they have shown that the carboxy-terminal half of the protein contains a basic repressor domain which can inhibit transcription from a promoter at a distance, consistent with the view that MeCP2 interacts with the transcriptional machinery or the initiation complex, rather than interfering with the binding of specific transcription factors. This suggests a mechanism

Figure 1



A model of how methylation may cause repression. The top shows nonmethylated, active chromatin with an activator (yellow) and transcription complex (green) engaged. Methylation modifies the chromatin structure to induce an inactive state. This inactive chromatin is resistant to activators and does not support transcription. MeCP2 (red) can bind to the methylated DNA directly, and has a repressor domain that can interfere with the transcriptional machinery at a distance.

by which methylated CpGs target the binding of proteins that contain repression domains and thereby inhibit expression. Many DNA-binding factors have difficulty accessing DNA when it is packaged in chromatin. In order for MeCP2 to repress expression from methylated genes, it has to be able to bind the DNA in the form that it is found *in vivo*, as chromatin. Indeed, MeCP2 has been shown to access sites on chromatin directly, suggesting that the binding of MeCP2 may stabilize and/or maintain the inactive chromatin.

A third strategy by which methylation may cause repression is by altering chromatin structure (Fig. 1). It has been shown that methylated DNA affects the positioning of nucleosomes and influences the sensitivity of the DNA to DNase I [14]. Further experiments using microinjection of methylated and nonmethylated templates into nuclei have shown that methylation inhibits expression only after chromatin is assembled [15]. These results support the view that methylation induces a change in conformation of chromatin to an inactive state. Kass *et al.* [16] have now elucidated further details of this mechanism. They have found that, after injection into *Xenopus* oocyte nuclei, both methylated and unmethylated templates are initially equally active. After longer incubation times, however, the methylated DNA is converted to an inactive form characterized by the loss of DNaseI hypersensitivity and of engaged RNA polymerase, and by the assembly of the inactivated promoter into a nucleosomal array. These results are consistent with the idea that the methylated DNA is assembled into a chromatin structure that inhibits transcription.

In vitro studies of chromatin formation have identified two levels of repression of nonmethylated templates. The assembly of nucleosome cores alone is able to bring about repression of basal transcription, but at lower nucleosome densities, such as those found in mammalian nuclei, histone H1 — the 'linker' histone between the cores — is crucial for complete repression. The addition of a strong activator, such as GAL4–VP16, can only counteract the repression caused by the addition of histone H1 [17]. Kass *et al.* [16] have found that, in contrast to unmethylated DNA, GAL4–VP16 cannot counteract the effect of chromatin once it has assumed the inactive state induced by DNA methylation. In this way, methylation not only stabilizes the inactive state but also prevents activation by blocking transcription factors.

Once the repressed state is established, the next question is how to activate an inactive gene. During development, stage-specific genes need to be activated at specific times. As these genes are presumably methylated and in an inactive chromatin conformation, how is activation accomplished? In light of the findings of Nan *et al.* [11] and Kass *et al.* [16], it appears that two events must occur before these genes may be activated: firstly, the methylation must be removed; and secondly, changes in chromatin must occur. It has already been shown that demethylation is necessary for gene expression [18]: now, the molecular mechanisms are becoming clear. Demethylation will cause the removal of specific factors that bind methylated DNA, such as MeCP2, and thus allow the destabilization of the chromatin. Once the chromatin is in a more accessible conformation, the binding of specific complexes such as SWI/SNF can relieve nucleosomal inhibition.

In conclusion, the methylation of CpG residues in the genome maintains and stabilizes the inactive state of chromatin. When chromatin is in this conformation, no expression can occur. In order to activate a gene that is in this state, the DNA has first to be demethylated, allowing the reversal of the inactive state of the chromatin and transcription to occur.

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